

- 21 -

Claims

1. A method for identification and speciation of bacteria of the *Burkholderia cepacia* complex in a sample, comprising the steps of

- (a) obtaining nucleotide sequence information for the *recA* gene in bacteria of the *Burkholderia cepacia* complex found in the sample; and
- (b) comparing the nucleotide sequence information obtained for the *recA* gene in bacteria of the *Burkholderia cepacia* complex found in the sample with a standard library of nucleotide sequence information comprising standard nucleotide sequence information for at least three species of bacteria of the *Burkholderia cepacia* complex.

2. The method of claim 1, wherein the nucleotide sequence information for bacteria of the *Burkholderia cepacia* complex in the sample and in the standard library are obtained by evaluation of restriction fragment length polymorphism.

3. The method of claim 2, wherein the restriction fragment polymorphism is carried out using the restriction enzyme *HaeIII* or *AluI*.

4. The method of ^{claim 1} ~~any of claims 1 to 3~~, wherein the *recA* gene in the bacteria of the *Burkholderia cepacia* complex in the sample is amplified relative to other nucleic acid polymers in the sample prior to obtaining the nucleotide sequence information.

5. The method of claim 4, wherein the *recA* gene is amplified using PCR amplification.

6. The method of claim 5, wherein the PCR amplification is carried out using the following primers:

Forward Primer

TGACCGCCGAGAAGAGCAA

Reverse Primer

SEQ ID No. 3

09763298.02004

Q

EPO - Munich

33

02 Sep. 2000

- 22 -

CTCTTCTTCGTCCATCGCCTC.

SEQ ID No. 4

7. The method of claim 5, wherein the PCR amplification is carried out using the following primers:

5 Forward Primer

TGCGGATGGGCGACGGCG

SEQ ID No. 20

Reverse Primer

CAGTTCTGTCGCTTGATCG.

SEQ ID No. 21

10 8. A composition comprising a pair of polynucleotide primers for production of a diagnostic amplicon from the recA gene of bacteria that is a member of the *Burkholderia cepacia* complex, said pair of primers hybridizing with each of the polynucleotides whose sequences are given by Seq. ID. Nos. 1, 2 and 5-19 to produce as an amplification product a diagnostic amplicon which can provide diagnostic information concerning the member of *Burkholderia cepacia* complex.

15 9. The composition of claim 8, wherein the polynucleotide primers have the sequences:

Forward Primer

TGACCGCCGAGAAGAGCAA

SEQ ID No. 3

Reverse Primer

CTCTTCTTCGTCCATCGCCTC.

SEQ ID No. 4

20 10. The composition of claim 8, wherein the polynucleotide primers have the sequence:

Forward Primer

TGCGGATGGGCGACGGCG

SEQ ID No. 20

Reverse Primer

CAGTTCTGTCGCTTGATCG.

SEQ ID No. 21

30

02 Sep. 2000

- 23 -

11. A kit for speciation of bacteria of the *Burkholderia cepacia* complex,
comprising, in packaged combination, a pair of polynucleotide primers in accordance with
claim 8, any of claims 8-10, and a discriminatory restriction endonuclease.

12. The kit of claim 11, wherein the restriction endonuclease is *HaeIII* or *AluI*.

13. A composition comprising a genomovar-specific primer pair effective
under stringent PCR conditions to produce amplification products by amplification of at
least a portion of the *recA* gene of bacteria belonging to one genomovar of the *B. cepacia*
complex, but not to produce amplification products from bacteria belonging to other
genomovars.

14. The composition according to claim 13, wherein the genomovar-specific
primer pairs are selected from among the following primer pairs given by Seq ID Nos.:
23 and 24, 25 and 26, 27 and 28, 29 and 30, 31 and 32, or 33 and 34.

15. A kit for speciation of bacteria of the *Burkholderia cepacia* complex,
comprising, in packaged combination, a pair of genomovar-specific polynucleotide
primers in accordance with claim 13 or 14 and a discriminatory restriction endonuclease.

16. The kit of claim 15, wherein the restriction endonuclease is *HaeIII* or *AluI*.

17. A vaccine composition for treatment and prevention of infection with
bacteria of the *Burkholderia cepacia* complex, wherein the bacteria is a member of
genomovar III and has a nucleotide sequence for the *recA* gene which produces a G-type
RFLP pattern when analyzed with the restriction enzyme *HaeIII*, and wherein the vaccine
composition comprises flagellin or a flagellin-derived antigen or a polynucleotide
encoding flagellin or a flagellin-derived antigen, said flagellin or flagellin-derived antigen
being obtained from the bacteria that is a member of genomovar III and that has a
nucleotide sequence for the *recA* gene which produces a G-type RFLP pattern when
analyzed with the restriction enzyme *HaeIII*.

add A1